IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:

YANG ET AL.

Appln. No.: Continuation of 09/398,897 Previous Group Art Unit: 1647

Filed: December , 2001 Previous Examiner: R. Hayes

For: STABLE NEURAL STEM CELLS

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January 14, 2002

PRELIMINARY AMENDMENT

Assistant Commissioner
for Patents
Washington, DC 20231

Sir:

Kindly amend the above-referenced patent application as follows.

IN THE SPECIFICATION:

On page 1, after the Title, delete:

"This patent application is directly related to U.S. provisional patent application 60/101,354, filed September 22, 1998, and is a continuation-in-part of U.S. patent application 09/053,414, filed April 1, 1998, now pending,

which is a continuation-in-part of U.S. patent application 08/719,450, filed September 25, 1996, now U.S. Patent 5,753,506, the entire contents of each is hereby incorporated by reference and relied upon."

Replace with:

--This patent application is a continuation of U.S. patent application 09/398,897, filed September 20, 1999, now pending, and is directly related to U.S. provisional patent application 60/101,354, filed September 22, 1998, and is a continuation-in-part of U.S. patent application 09/053,414, filed April 1, 1998, abandoned, which is a continuation-in-part of U.S. patent application 08/719,450, filed September 25, 1996, now U.S. Patent 5,753,506, the entire contents of each is hereby incorporated by reference and relied upon.--

IN THE CLAIMS:

Kindly cancel claims 1-5 and 7-22.

Kindly add new claims 23-38, as follows:

--23. An *in vitro* stable cell line of mammalian neural precursor cells,

wherein the mammalian neural precursor cells contain a c-myc construct and

wherein the c-myc construct is comprised of a c-myc cDNA fused with at least one element selected from the group consisting of DNA for a ligand binding domain for an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, a thyroid hormone receptor, a retinoid receptor, and an ecdysone receptor.

24. The cell line of claim 23, wherein the mammalian neural precursor cells are derived from a human.

25. The cell line of claim 23, wherein the mammalian neural precursor cells are derived from an *in vitro* culture of pluripotent embryonic stem cells.

26. The cell line of claim 23, wherein the cells maintain a multipotential capacity to differentiate into neurons, astrocytes and oligodendrocytes.

27. The cell line of claim 23, wherein the cells maintain a bipotential capacity to differentiate into neurons and astrocytes.

28. The cell line of claim 23, wherein the cells maintain a bipotential capacity to differentiate into astrocytes and oligodendrocytes.

29. The cell line of claim 23, wherein the cells maintain a unipotential capacity to differentiate into neurons.

30. The cell line of claim 23, wherein the cells maintain a unipotential capacity to differentiate into astrocytes.

31. An *in vitro* stable cell line of mammalian neural precursor cells, produced by:

a) preparing a culture of neural precursor cells in a serum-free medium;

b) culturing the neural precursor cells in the presence of a first mitogen, wherein said first mitogen is

selected from the group consisting of aFGF, bFGF, EGF, TGFα and combinations thereof;

c) introducing a c-myc construct into the cells,

wherein the c-myc construct is comprised of a c-myc cDNA fused with at least one element selected from the group consisting of DNA for a ligand binding domain for an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, a thyroid hormone receptor, a retinoid receptor, and an ecdysone receptor; and

d) further culturing the cells in a medium containing the first mitogen and a second mitogen,

wherein said second mitogen is selected from the group consisting of aFGF, bFGF, EGF, TGFα, serum and combinations thereof, with the proviso that the second mitogen is other than the first mitogen, and

wherein said medium containing the first mitogen and the second mitogen further comprises a myc-activating chemical selected from the group consisting of β-estradiol, RU38486, dexamethasone, thyroid hormones, retinoids, and ecdysone.

32. The cell line of claim 31, wherein the mammalian neural precursor cells are derived from a human.

33. The cell line of claim 31, wherein the mammalian neural precursor cells are derived from an *in vitro* culture of pluripotent embryonic stem cells.

34. The cell line of claim 31, wherein the cells maintain a multipotential capacity to differentiate into neurons, astrocytes and oligodendrocytes.

35. The cell line of claim 31, wherein the cells maintain a bipotential capacity to differentiate into neurons and astrocytes.

36. The cell line of claim 31, wherein the cells maintain a bipotential capacity to differentiate into astrocytes and oligodendrocytes.

37. The cell line of claim 31, wherein the cells maintain a unipotential capacity to differentiate into neurons.

38. The cell line of claim 31, wherein the cells maintain a unipotential capacity to differentiate into astrocytes.--

REMARKS

The above-referenced continuation application has been preliminarily amended by updating the prosecution history of the patent applications, by canceling claims 1-5 and 7-22 and by adding new claims 23-38. Replacement page 1 and a clean copy of the claims after amendment are included in the attached Appendix.

More specifically, page 1 of the specification has been amended by updating the prosecution history of the patent applications to show that the present application is a continuation of U.S. patent application 09/398,897 and to show that parent patent application 09/053,414 has been abandoned. New claims 23-38 directly correspond to claim 6 and canceled claims 7-11 of the present application.

Thus, no new matter has been added by any of these amendments.

Early and favorable action on the merits are respectfully requested.

Should any matters remain in this application which might be resolved by interview, the Examiner is requested to telephone the undersigned at (570) 386-5744.

Respectfully submitted,

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APPENDIX

Replacement Page

Stable Neural Stem Cell Lines

This patent application is a continuation of U.S. patent application 09/398,897, filed September 20, 1999, now pending and is directly related to U.S. provisional patent application 60/101,354, filed September 22, 1998, and is a continuation-in-part of U.S. patent application 09/053,414, filed April 1, 1998, abandoned, which is a continuation-in-part of U.S. patent application 08/719,450, filed September 25, 1996, now U.S. Patent 5,753,506, the entire contents of each is hereby incorporated by reference and relied upon.

Background of the Invention

1. FIELD OF THE INVENTION

The present application discloses a systematic and efficient method for establishing stable neural stem cell lines and neuronal progenitor lines. The resulting cell lines provide robust, simple, and reproducible cultures of human and other mammalian neurons in commercially useful mass quantities while maintaining normal karyotypes and normal neuronal phenotypes.

2. DESCRIPTION OF THE RELATED ART

A developing fetal brain contains all of the cells germinal to the cells of an adult brain as well as all of the programs necessary to orchestrate them toward the final network of neurons. At early stages of development, the nervous system is populated by germinal cells from which all other cells, mainly neurons, astrocytes and oligodendrocytes, derive during subsequent stages of development. Clearly such

CLAIMS AFTER AMENDMENT

6. A cell line produced according to the method of claim 1.

23. An *in vitro* stable cell line of mammalian neural precursor cells,

wherein the mammalian neural precursor cells contain a c-myc construct and

wherein the c-myc construct is comprised of a c-myc cDNA fused with at least one element selected from the group consisting of DNA for a ligand binding domain for an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, a thyroid hormone receptor, a retinoid receptor, and an ecdysone receptor.

24. The cell line of claim 23, wherein the mammalian neural precursor cells are derived from a human.

25. The cell line of claim 23, wherein the mammalian neural precursor cells are derived from an *in vitro* culture of pluripotent embryonic stem cells.

26. The cell line of claim 23, wherein the cells maintain a multipotential capacity to differentiate into neurons, astrocytes and oligodendrocytes.

27. The cell line of claim 23, wherein the cells maintain a bipotential capacity to differentiate into neurons and astrocytes.

28. The cell line of claim 23, wherein the cells maintain a bipotential capacity to differentiate into astrocytes and oligodendrocytes.

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a) preparing a culture of neural precursor cells in a serum-free medium;

b) culturing the neural precursor cells in the presence of a first mitogen, wherein said first mitogen is selected from the group consisting of aFGF, bFGF, EGF, TGFα and combinations thereof;

c) introducing a c-myc construct into the cells,

wherein the c-myc construct is comprised of a c-myc cDNA fused with at least one element selected from the group consisting of DNA for a ligand binding domain for an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, a thyroid hormone receptor, a retinoid receptor, and an ecdysone receptor; and

d) further culturing the cells in a medium containing the first mitogen and a second mitogen,

wherein said second mitogen is selected from the group consisting of aFGF, bFGF, EGF, TGFα, serum and combinations thereof, with the proviso that the second mitogen is other than the first mitogen, and

wherein said medium containing the first mitogen and the second mitogen further comprises a myc-activating

chemical selected from the group consisting of β-estradiol, RU38486, dexamethasone, thyroid hormones, retinoids, and ecdysone.

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